

Certain Aspects related to the Feeding of Animal Proteins to Farm Animals¹

Scientific Opinion of the Panel on Biological Hazards

(Question N° EFSA-Q-2007-084)

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TABLE OF CONTENTS

Panel Members.....	1
Table of contents.....	2
1 SUMMARY.....	3
2 BACKGROUND.....	4
2.1 Introduction.....	4
2.2 Background to the Mandate.....	5
3 TERMS OF REFERENCE.....	5
4 ACKNOWLEDGEMENTS.....	6
5 ASSESSMENT.....	6
6 CONCLUSIONS.....	6
7 ANSWERS TO TERMS OF REFERENCE.....	8
7.1 First Term of Reference.....	8
7.2 Second Term of Reference.....	8
7.3 Implications between the first and the second Terms of Reference.....	9
8 RECOMMENDATIONS.....	9
9 DOCUMENTATION PROVIDED TO EFSA.....	9
10 REFERENCES.....	10
APPENDIX A.....	11
APPENDIX B.....	37
GLOSSARY.....	39

1 SUMMARY

The European Food Safety Authority (EFSA) was requested by the European Parliament (EP) to assess, with respect to Bovine Spongiform Encephalopathy (BSE), the safety for human health of the utilisation of non-ruminant Meat and Bone Meal (MBM). More specifically, EFSA was requested (i) to evaluate the risk from using non-ruminant MBM in pig and poultry feed, once it is possible to distinguish protein origin up to different species and (ii) the introduction of certain tolerance levels with regard to small quantities of MBM in animal feed and the parameters which could be utilized to define these tolerance levels and quantities.

The EFSA opinion takes account of the general control measures in place in the European Union (EU) and assumes the effectiveness of these controls in avoiding cross-contamination, both deliberate and accidental. This opinion considers all available scientific data and information related to the risk of transmission of the BSE agent through feed and, by this means, addresses the risk of causing BSE related exposure to humans, as well as risks related to some other TSE agents. In replying to the above mentioned questions, this assessment only considers the use of pig Processed Animal Proteins² (PAPs) in poultry feed and the use of poultry PAPs in pig feed. With respect to the introduction of certain tolerance levels with regards to small quantities of MBM in animal feed, this assessment considers such a tolerance for animal proteins of any species in animal feed.

To date, no Transmissible Spongiform Encephalopathies (TSEs) have been identified as occurring in pigs or poultry under natural conditions. Taking account of the epidemiological situation of BSE in cattle in the EU, which indicates a decreasing trend, together with the current control measures in place to avoid exposure of pigs and poultry to BSE contaminated material, the EFSA Scientific Panel on Biological Hazards (BIOHAZ) concluded that the risk of transmitting BSE to pigs utilizing poultry PAPs and *vice versa* is negligible. Consequently in this scenario any increase in the exposure risk of BSE to humans would be negligible. If TSE in birds or pigs is identified in the future as occurring under natural conditions, the assessment presented here will no longer be valid.

The BIOHAZ Panel further concluded that the risk of transmitting BSE through small quantities of animal proteins in feed to ruminants can not be excluded, but considering the current protective measures in place in the EU³, the few infected animals that could arise from this contamination would probably not be able to sustain the BSE epidemic but would increase the human exposure risk to BSE. The risk of transmitting BSE to non-ruminants is considered to be lower than to ruminants, as long as intra-species recycling is avoided. Consequently in this scenario the increase in the exposure risk of BSE to humans is negligible.

In the event that a tolerance level was required to be set up in order to quantify animal proteins in animal feed, the BIOHAZ Panel considered the Limit of Quantification (LOQ) of

² In Commission Regulation (EC) No 829/2007 of 28 June 2007 is defined as: “*animal protein derived entirely from Category 3 material, which have been treated in accordance with Chapter II of Annex VII so as to render them suitable for direct use as feed material or for any other use in feedingstuffs, including petfood, or for use in organic fertilisers or soil improvers; however, it does not include blood products, milk, milk-based products, colostrum, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, tricalcium phosphate and collagen*”.

³ Regulation (EC) 999/2001 as amended and Regulation (EC) 1774/2002 as amended.

the method used to set such tolerance level as the parameter required. However the BIOHAZ Panel concluded that it is currently not possible to set a LOQ because of insufficient data on the performance of relevant detection methods for quantification. It is therefore recommended that studies be conducted to define the LOQ for different types of animal proteins in feed.

In a hypothetical situation in which pigs are allowed to be fed with poultry PAPs and *vice versa* or, in general, inter-species recycling is allowed, currently it is not possible to quantify the level of contamination with non authorized products containing animal proteins in feed. Accordingly it is technically not possible at present to determine whether the contamination is below or above a defined tolerance level.

The BIOHAZ Panel further concluded that compared to the current measures in place in EU, the introduction of a tolerance level, which has to be defined at a certain level above the LOQ, will lead to an increase in the risk of transmission of BSE or other TSEs, depending on the species. This increased risk can not be quantified.

Key Words: Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathies (TSEs), Meat and Bone Meal (MBM), Processed Animal Proteins (PAPs), Pig, Poultry, Tolerance Level

2 BACKGROUND

2.1 Introduction

Since Bovine Spongiform Encephalopathy (BSE) was reported for the first time in 1986 in the United Kingdom (UK), the European Union (EU) has developed a comprehensive set of risk reducing measures on Transmissible Spongiform Encephalopathies (TSEs) in order to protect human health from BSE and to control and eventually eradicate TSEs in animals. The key piece of legislation to protect human and animal health from the risk of BSE and other TSEs is Reg. (EC) 999/2001 (EC, 2001) laying down rules for the prevention, control and eradication of certain TSEs. This legislation is continuously reviewed in the light of new scientific evidence, the evolution of the TSE situation and the practical implementation in the field.

One of the most effective risk reducing measures consisted of a total EU wide ban on the use of animal protein in feeds for any animal farmed for the production of food, with some exceptions (*e.g.* use of fishmeal in non-ruminants). This measure was introduced in January 2001.

Since the implementation of the TSE Regulation in 2001, more than 61 million adult bovine animals have been tested across the EU and around 7400 cases have been detected. A constant decline (about 30% per year) in the number of cases has been recorded: from 2167 cases in 2001 to 320 cases in 2006. Out of this only 27 cases were related to animals born after the start of the total feed ban as mentioned above.

Feed microscopy is currently the only method officially endorsed by the European Community to test for the presence of animal protein in feeds. However, recently new methods, *i.e.* Polymerase Chain Reaction (PCR), immuno-assays and Near Infrared Microspectrometry (NIRM) are under development and validation, and could in principle identify the species composition of products containing animal proteins.

2.2 *Background to the Mandate*

On 12th September 2006, the European Economic and Social Committee (EESC) adopted an opinion in which it suggests ‘*that the European Commission pursue and step up as swiftly as possible the studies currently under way which clearly show that the use of meat meal from non-ruminants can be used in pig and poultry feed without posing any danger to human health*’ (EESC, 2006).

On 13th December 2006, the European Parliament adopted, in agreement with the Council, a regulation amending Regulation (EC) No 999/2001 for the prevention, control and eradication of certain TSEs (EC, 2006). In the absence of scientific data on the distinction between proteins of different species, this regulation does not amend the rules in force regarding the use of meat and bone meal from non-ruminants in pig and poultry feed.

Moreover, in its working document on legislative rules in the TSE field 2006 – 2007, the European Commission announced discussions on the introduction of certain tolerance levels with regard to small quantities of meat and bone meal in animal feed (EC, 2006).

Against this background, and in accordance with Article 119 of the Rules of Procedure of the European Parliament, the European Food Safety Authority (EFSA) was invited to provide an opinion, with respect to the risk of BSE, on the use of meat and bone meal from non-ruminants in pig and poultry feed, taking account of the ban on intra-species recycling.

3 TERMS OF REFERENCE

The European Food Safety Authority is invited by the European Parliament to provide an opinion on the following questions:

- Is the use of meat and bone meal from non-ruminants in pig and poultry feed possible without posing any danger, with respect to BSE, to human health once it is possible to distinguish proteins according to different species?
- Is the concept of the introduction of certain tolerance levels, with regard to small quantities of meat and bone meal in animal feed, sound enough in scientific terms to decide on the potential dangers, with respect to BSE, to human health? What parameters would be used to define these tolerance levels and quantities?

Clarifications on the Terms of Reference

The opinion and its risk assessment contained herein, takes account of the general control measures in place in the EU and assumes the effectiveness of these controls in avoiding cross-contamination, both deliberate and accidental.

It was noted that the mandate as received from the EU Parliament did not specifically request to address the BSE-related risk. However, the introductory part of the mandate seemed suggesting that the remit of the mandate was related to BSE. Based on communication between the EFSA and the EU Parliament, it was clarified that the scope of the mandate concerned only BSE-related risks. Therefore, the opinion and its risk assessment contained herein, include the risk of transmission of the BSE agent through feed and the consequent risk of causing BSE related exposure in humans, as well the risk related to other TSE agents.

The first Term of Reference mentions using Meat and Bone Meal⁴ (MBM) from non-ruminants in pig and poultry feed. The context of this opinion and its risk assessment is focused on the use of Processed Animal Proteins⁵ (PAPs) from pig origin in poultry feed and the use of PAPs from poultry origin in pig feed. This is based on the common practice of using almost exclusively proteins from these non-ruminant species (pig and poultry) in feedstuffs. Moreover, in the opinion of the European Economic and Social Committee (EESC, 2006), on which the present mandate is largely based, the question is focussed on “*systems exclusively supplying pork protein for poultry feed and vice versa*”.

The second Term of Reference mentions the introduction of certain tolerance levels with respect to small quantities of MBM in animal feed. The context of this opinion and its risk assessment is widened to the introduction of a tolerance level for products containing animal proteins of any species in animal feed.

4 ACKNOWLEDGEMENTS

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5 ASSESSMENT

Please refer to the Scientific Report in Appendix A

6 CONCLUSIONS

- There is scientific evidence that pigs are susceptible to BSE when exposed parenterally.
- Even recognising that significant amounts of BSE infectivity have been fed to pigs in the UK and additionally that intra-species pig to pig recycling could have happened, no naturally occurring TSE, including BSE, have been detected so far in pigs. However, the scarcity of available data does not allow conclusive evidence on the absence of TSE in pigs. It is therefore concluded that if TSE was present in pigs it would have been at low prevalence.

⁴ In Commission Directive 92/87/EEC of 26 October 1992 is defined as: “*Product obtained by heating, drying and grinding whole or parts of warm-blooded land animals from which the fat may have been partially extracted or physically removed. The product must be substantially free of hooves, horn, bristle, hair and feathers, as well as digestive tract content. (Minimum crude protein content 50 % on a dry matter basis)*”

⁵ In Commission Regulation (EC) No 829/2007 of 28 June 2007 is defined as: “*animal protein derived entirely from Category 3 material, which have been treated in accordance with Chapter II of Annex VII so as to render them suitable for direct use as feed material or for any other use in feedingstuffs, including petfood, or for use in organic fertilisers or soil improvers; however, it does not include blood products, milk, milk-based products, colostrum, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, tricalcium phosphate and collagen*”.

- Considering the current epidemiological situation of BSE in the EU and current control measures in place to avoid the exposure of pigs to BSE-contaminated material, the risk of transmitting BSE to poultry fed with feed containing pig PAPs is negligible. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- Should TSE be identified in pigs under natural conditions this assessment will no longer be valid.
- According to current knowledge, the risk of transmitting BSE to birds is considered negligible. Moreover, there is no evidence of active replication of the BSE agent in birds after oral or parenteral exposure. Up to date, no naturally occurring TSE, including BSE, have been detected in birds. However, the scarcity of available data does not allow the definite conclusion on the absence of TSE in birds.
- Considering the current epidemiological situation of BSE in the EU and the current control measures in place to avoid the exposure of poultry to BSE-contaminated material, the risk of transmitting BSE to pigs fed with feed containing poultry PAPs is negligible. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- Should TSE be identified in birds under natural conditions this assessment will no longer be valid.
- If pigs and poultry were fed with TSE-contaminated material the content of the gut and manure may present a risk because even the treatment at “133°C/20’/3 bars” may not eliminate all TSE infectivity if the initial titre was high.
- The transmission of BSE through small quantities of products containing BSE contaminated animal proteins, even when present below 0.1%, in feed to ruminants cannot be excluded. The few infected animals that could arise from such contamination would probably not be able to sustain the BSE epidemic but would increase the human exposure risk to BSE.
- The risk of transmitting BSE through small quantities of products containing BSE contaminated animal proteins, even when present below 0.1%, in feed to non-ruminants is considered to be lower than in ruminants, as long as intra-species recycling is avoided. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- PCR and immuno-assays are currently the only techniques available to distinguish the species origin of products containing animal proteins from different animal species. However, PCR can assign a species origin and give an indication of a possible contamination but is not able to distinguish products made from authorised or unauthorised tissues. Depending on the protein that is targeted, the same problem may exist with immunoassays. Such problems might be overcome by applying the detection method on the raw material or by using different analytical techniques on the same sample.
- The parameter required to set up a tolerance level is the Limit of Quantification (LOQ) of the method used to quantify products that are sources of animal protein in feed. However, it is currently not possible to set a LOQ because of insufficient data on the performance of relevant detection methods for quantification.
- Compared to the current measures in place in EU, the introduction of a tolerance level, which has to be defined at a certain level above the Limit of Quantification, will lead to an

increase in the risk of transmission of BSE or other TSEs depending on the species involved. This increased risk can not be quantified.

- If pigs are allowed to be fed with poultry PAPs and *vice versa* or, in general, inter-species recycling is allowed, currently it is not possible to quantify the level of contamination with non authorized products containing animal proteins in feed for these animals. Accordingly it is technically not possible at present to determine whether the contamination is below or above a defined tolerance level. For example the quantification of ruminant MBM at trace level in poultry feed containing 5% pig MBM is currently impossible.
- Even considering the progress in laboratory detection techniques (both in term of sensitivity and discrimination power), where (i) some raw animal by-products would circulate and (ii) tolerance level of contamination would be introduced, the application of a targeted sampling scheme could result in possible major cross-contamination incidents, the magnitude of which could remain un-identified.
- In a context where TSEs are still circulating in farm animal species, any intra-species recycling of animal products would increase the risk of TSE propagation.
- This assessment remains valid only in the context of the continuation of the other regulatory measures laid down in Reg. EC 999/2001 and 1774/2002 (ban on intra-species recycling, SRM removal and ban on proteins derived from ruminants).

7 ANSWERS TO TERMS OF REFERENCE

7.1 First Term of Reference

- Considering the current epidemiological situation of BSE in the EU and the current control measures in place to avoid the exposure of poultry to BSE-contaminated material, the risk of transmitting BSE to pigs fed with feed containing poultry PAPs is negligible. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- Considering the current epidemiological situation of BSE in the EU and current control measures in place to avoid the exposure of pigs to BSE-contaminated material, the risk of transmitting BSE to poultry fed with feed containing pig PAPs is negligible. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- Up to to-date, no TSE have been identified in pigs or poultry under natural conditions. If TSE in birds or pigs would be identified under natural conditions, this assessment will no longer be valid.

7.2 Second Term of Reference

- The transmission of BSE through small quantities of products containing BSE contaminated animal proteins, even when present below 0.1%, in feed to ruminants cannot be excluded. The few infected animals that could arise from such contamination would probably not be able to sustain the BSE epidemic but would increase the human exposure risk to BSE.

- The risk of transmitting BSE through small quantities of products containing BSE contaminated animal proteins, even when present below 0.1%, in feed to non-ruminants is considered to be lower than in ruminants, as long as intra-species recycling is avoided. Consequently, any increase in the exposure risk of BSE to humans is negligible.
- The parameter required to set up a tolerance level is the Limit of Quantification (LOQ) of the method used to quantify products that are sources of animal protein in feed. However, it is not currently possible to set a LOQ because of insufficient data on the performance of relevant detection methods for quantification.
- Compared to the current measures in place in EU, the introduction of a tolerance level, which has to be defined at a certain level above the Limit of Quantification, will lead to an increase in the risk of transmission of BSE or other TSEs depending on the species involved. This increased risk can not be quantified.

7.3 *Implications between the first and the second Terms of Reference*

- If pigs are allowed to be fed with poultry PAPs and *vice versa* or, in general, inter-species recycling is allowed, currently it is not possible to quantify the level of contamination with non authorized products containing animal proteins in feed for these animals. Accordingly it is technically not possible at present to determine whether the contamination is below or above a defined tolerance level. For example the quantification of ruminant MBM at trace level in poultry feed containing 5% pig MBM is currently impossible.

8 RECOMMENDATIONS

It is recommended to initiate studies to define the LOQ for different types of products containing animal proteins (MBM, Meat Meal...) in feed.

9 DOCUMENTATION PROVIDED TO EFSA

Letter (ref. n. 200773 date 14/02/2007) from the European Parliament with a request for an opinion on certain aspects related to the feeding of Animal Proteins to Farm Animals http://www.efsa.europa.eu/etc/medialib/efsa/science/biohaz/biohaz_requests_mandates/biohaz_rm_mbm.Par.0001.File.dat/request_letter.pdf

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APPENDIX A

**Scientific Assessment of the Panel on Biological Hazards on Certain Aspects
related to the Feeding of Animal Proteins to Farm Animals**

TABLE OF CONTENTS

Table of contents.....	12
1 Preamble.....	13
2 Scientific Background	14
2.1 Animal exposure under field conditions	14
2.2 Experimental BSE infections in Pigs	15
2.2.1 Oral challenge of pigs with the agent of cattle BSE.....	15
2.2.2 Oral challenge of pigs with passaged BSE and other TSE agents.....	16
2.2.3 Parenteral challenge of pigs with the agent of cattle BSE.....	16
2.3 Experimental TSE infections in transgenic mice	17
2.3.1 Oral challenge of Porcine-PrP transgenic mice with the agent of cattle BSE.	17
2.3.2 Intracerebral challenge of Porcine-PrP transgenic mice with the agent of cattle BSE.....	17
2.3.3 Intracerebral challenge of Porcine-PrP transgenic mice with the agent of sheep adapted BSE	18
2.3.4 Intracerebral challenge of Porcine-PrP transgenic mice with other TSE agents	18
2.3.5 The BSE transmission barrier to pigs.....	18
2.3.5.1 <i>The transmission barrier to humans for the agent of pig adapted BSE</i>	19
2.4 Natural BSE in Pigs	19
2.4.1 Historical exposure of pigs to TSE agents	19
2.4.2 Likelihood of identifying BSE clinically infected pigs under natural conditions.....	19
2.4.3 Possibility of spontaneously occurring TSE in pigs.....	21
2.4.4 TSE Surveillance in pigs.....	21
2.4.5 Risk of residual infectivity after exposure to BSE contaminated feed.....	22
2.5 TSE Infection in pigs: conclusion on the current scientific information.....	22
2.6 BSE infection in birds.....	23
2.7 Current discriminatory tests for products containing animal proteins in feed.....	24
2.7.1 Species-specific detection	25
2.7.1.1 <i>Classical microscopy and Near Infrared Microspectrometry (NIRM)</i>	25
2.7.1.2 <i>Immunoassay</i>	26
2.7.1.3 <i>PCR</i>	26
2.7.2 Tolerance level	27
2.7.2.1 <i>Classical microscopy and Near Infrared Microspectrometry (NIRM)</i>	28
2.7.3 Limitation of setting tolerance levels in the event of poultry PAPs being allowed to be fed to pigs and vice versa	29
3 Risk Assessment.....	30
3.1 BSE Risk deriving from the utilization of poultry PAPs in pig feed	30
3.2 BSE Risk deriving from the utilization of pig PAPs in poultry feed	30
3.3 Risk of transmitting BSE through small quantities of products containing animal proteins in feed..	31

3.3.1	Risk of transmitting BSE through small quantities of products containing animal proteins in feed to ruminants	31
3.3.2	Risk of transmitting BSE through small quantities of products containing animal proteins in feed to non-ruminants.....	32
4	References	33
	APPENDIX B.....	37
	GLOSSARY	39

1 Preamble

This assessment takes account of the general control measures in place in the EU and assumes the effectiveness of these controls in avoiding cross-contamination, both deliberate and accidental. However, the limitations of the present test methods used in the frame of these controls and a certain level of non-compliance might be taken into account when applying this document.

The use of MBM, meat meal⁶ (MM) and blood meal in diets for pigs and poultry was common practice in many countries in the decades before BSE was detected in 1986 in the UK. By the 1980's the average inclusion rate of MBM in pig feeds was 5% with a usage in excess of 175,000 tonnes per year of meat derived products in pig diets in the UK.

The ban on the use of ruminant protein in ruminant feed in the UK in July 1988 (Anon, 1988) was the result of concern about intra-species recycling. Also in the UK, between 1990 and 1996, some feed companies stopped using animal proteins, other than fishmeal and milk products, in feeds for pigs and poultry. Others continued to use these ingredients until the use of mammalian proteins in livestock feed was banned in 1996. Despite the 1996 ban in the UK, the feeding of non-ruminant mammalian meat and bone meal to pigs and poultry remained legal in other countries of the EU. Nevertheless, sentiment and market forces contributed to a marked decline in its use in the last few years of the 1990's. Following the introduction in 1994 of the EU ban on mammalian protein in ruminant feeds, many feed mills that manufactured both ruminant and non-ruminant animal feeds, ceased the use of animal proteins in any feeds.

Since January 2001 the use of all processed animal protein in feeds for farmed animals has been banned throughout the EU with some exceptions (i.e. fish meal for non-ruminants; tuber, root crops and feedingstuffs containing such products following the detection of bone spicules after a favourable risk assessment), but its use in other parts of the world continues.

⁶ In Commission Directive 92/87/EEC of 26 October 1992 is defined as: “*Product obtained by heating, drying and grinding whole or parts of warm-blooded land animals from which the fat may have been partially extracted or physically removed. The product must be substantially free of hooves, horn, bristle, hair and feathers, as well as digestive tract content. (Minimum crude protein content 50 % on a dry matter basis)*”.

In the EU the use of proteins derived from animals in animal nutrition is regulated by two pieces of legislations, namely the Regulation (EC) No 1774/2002 (Animal By-Products Regulation) (EC, 2002) and the Regulation (EC) No 999/2001 (TSE Regulation) (EC, 2001).

Regulation (EC) 1774/2002 of the European Parliament and the Council lays down health rules concerning animal by-products not intended for human consumption. The key is the exclusion of dead animals and other condemned materials from the feed chain. Under this Regulation, only materials derived from animals declared fit for human consumption following veterinary inspection may be used for the production of feeds. It also bans intra-species recycling. This Regulation shall not affect veterinary legislation having as its objective the eradication and control of certain diseases.

Regulation (EC) No 999/2001 is the key piece of legislation to protect human and animal health from the risk of BSE and other TSEs. Regarding the feeding provisions an EU wide ban on the feeding of proteins derived from animals to ruminants was in place since July 1994. This ban was extended to a suspension on the use of all proteins derived from animals in feeds for any animals farmed for the production of food since 1 January 2001 with some exceptions (i.e. fish meal for non-ruminants; tuber, root crops and feedingstuffs containing such products following the detection of bone spicules after a favourable risk assessment).

The use of proteins derived from animals in animal nutrition as foreseen by Regulation (EC) No 1774/2002 is overruled by the more stricter rules laid down in the TSE Regulation (EC) No 999/2001 introducing an EU wide ban on the use of proteins derived from animals as feed ingredient for all farmed animals.

A number of relevant Opinions of the Scientific Steering Committee (SSC) of the European Commission were taken into account in the present assessment. These concern the potential for TSE agents to occur in the tissues of non-ruminant livestock species, including pigs and poultry, and the possible risks associated with the entry of such animal proteins potentially contaminated with TSE agents into the food or feed chains and the recycling of TSE infectivity in animal feed.

A quantitative risk assessment has been carried out by EFSA on “the animal BSE risk posed by meat and bone meal with respect to the residual BSE risk ” (EFSA, 2005), and the outcome supported the conclusions of the above mentioned SSC opinions on MBM.

2 Scientific Background

2.1 Animal exposure under field conditions

The exact modalities of animal exposure to TSEs under field conditions is currently unknown. In particular, the consequences of a single effective dose or repeated minimal doses on contamination efficacy and animal susceptibility is unclear.

Diringer and Jacquemot found some circumstantial evidence for a cumulative dose effect of TSE infectivity by observing a distinctly higher infection rate in groups of mice and hamsters dosed repeatedly with low doses (below the ID₅₀⁷) of scrapie than in groups dosed once with the same low dose (Diringer *et al.*, 1998; Jacquemot *et al.*, 2005).

⁷ ID₅₀: Infectious Dose 50, the dose which infects 50% of animals in an experimental test.

To date most of the experiments conducted in order to demonstrate transmissibility of TSEs in species of interest (like cattle or pigs with BSE) consisted in a single administration of generally high infectious dose. In fact, field occurrence of BSE seems to be consistent with single low dose exposure (Wells *et al.*, 2007). However, relevance of the results of single-dose studies in terms of susceptibility have to be considered with some caution, although there is some evidence that a single dose exposure was the most likely mean of infection

2.2 Experimental BSE infections in Pigs

2.2.1 Oral challenge of pigs with the agent of cattle BSE.

Two different studies were undertaken in the EU in order to investigate the oral transmissibility of the BSE agent to pigs.

In the first study, performed in the UK, ten eight weeks old pigs were orally challenged with a total of 4 kg of brain material from clinically BSE affected cattle (infectious titer of the inoculum: $10^{2.4}$ i.c./i.p. mouse LD₅₀/g⁸) on three successive occasions, at 1-2 week intervals (Wells *et al.*, 2003).

Assuming that equal amounts were ingested, each pig:

- consumed about 400g (about 10^5 i.c./i.p. mouse LD₅₀) of infected tissue on each occasion, which is equivalent to the maximum daily intake of meat and bone meal in rations for pigs aged 8 weeks;
- was exposed to 1.2 kg (about $10^{5.5}$ i.c./i.p. mouse LD₅₀) of infected brain tissue in total.

In cattle orally challenged with dilution series of BSE cattle material (Wells *et al.*, 2007) a transmission (1 out of 15 challenged individuals) was observed after challenge with $10^{0.5}$ i.c./i.p. mouse LD₅₀ and the cattle oral LD₅₀ was estimated to be equivalent to $10^{2.8}$ i.c./i.p. mouse LD₅₀ (95% C.I. $10^{2.0} - 10^{3.5}$).

Consequently, in this experiment pigs were orally exposed to a total dose that would be equivalent to 500 oral LD_{50s} in cattle.

No clinical or pathological evidence of TSE was found in the pigs up to seven years post-challenge. No infectivity was detected in two sets of pools composed with single tissues; one from 5 exposed pigs ranging from 80 to 109 weeks post-exposure assayed in C57BLJ6 mice and the other one from 3 exposed pigs ranging from 350 to 371 weeks post-exposure assayed in RIII mice. The tissues assayed were: brain, spinal cord, semitendinosus muscle, spleen, thymus, retropharyngeal, mesenteric and popliteal lymph nodes, stomach, distal ileum, pancreas, liver and kidney. However, the results of this infectivity study have to be interpreted with caution because of the low sensitivity of the detection bioassay used.

In a second experiment, which was part of an EU project (FAIR CT97-3306), six LargeWhite x Landrace pigs aged nine weeks were inoculated by the oral route with a dose of 75 g (3 animals), or 3 doses of 75 g (3 animals) administrated at 0, 7 and 14 days from the first dose. Inoculum consisted of an untitred pool of BSE material (TSE/08/59) originating from the brainstem of 49 BSE affected cattle supplied by the Veterinary Laboratory Agency, New Haw, Addlestone, Surrey, U.K.) and homogenised in PBS at 1:10. No clinical or pathological

⁸ LD₅₀: Lethal Dose 50, the dose which kills 50% of animals in an experimental test.

evidence of TSE was found in the pigs up to 33 months post-challenge. No infectivity was found in brain from the pigs exposed orally when assayed in PoPrP-Tg001 mice. However, the results obtained in this study must be interpreted with caution as the observation time was too short to exclude later manifestations of the infection and the infectivity of the inoculum was probably lower than in the first study.

2.2.2 Oral challenge of pigs with passaged BSE and other TSE agents

To date there is no information available concerning experimental oral challenge in pigs with:

- (i) BSE sub-passaged in another species (small ruminants)
- (ii) BSE adapted to pigs; which would be relevant for assessment of transmission risk of BSE in pigs in case of intra-species recycling
- (iii) TSE agents other than BSE

2.2.3 Parenteral challenge of pigs with the agent of cattle BSE

Two different studies were undertaken in EU in order to investigate the transmissibility of the BSE agent to pigs.

The first study began in the UK in 1989 (Wells *et al.*, 2003). Ten pigs aged 1-2 weeks were challenged with an unclarified 10% (w/v) saline suspension of a pool of homogenized brain stems from four natural, histopathologically confirmed BSE cases (infectious titer: $10^{2.4}$ i.c./i.p. mouse LD₅₀/g) by three routes simultaneously (intracranially [i.c.] 10^2 i.c./i.p. mouse LD₅₀, intravenously [i.v.] 1 to 2 $10^{2.4}$ i.c./i.p. mouse LD₅₀ and intraperitoneally [i.p.] with about $10^{3.2}$ to i.c./i.p. mouse LD₅₀).

- Two animals died of intercurrent disease at 47 and 50 weeks post challenge. Neither clinical signs or PrP^{Sc} accumulation in CNS were observed in these animals.
- One animal developed clinical neurological signs and was killed at 74 weeks post-inoculation (p.i.) with a spongiform encephalopathy and the presence of PrP^{Sc} in the brain.
- Three apparently healthy animals were killed electively between 105 and 107 weeks p.i. In two out of these three pigs pre-clinical pathological (vacuolar) changes and PrP^{Sc} were detected.
- In the remaining 4 animals killed at 139-163 weeks p.i., clinical signs and TSE was confirmed.

In this experiment 7 out of 8 pigs manifested clinical (estimated incubation period range: 69 - 150 weeks) or subclinical infection.

Infectivity was detected by bioassay in inbred mice in the central nervous system (brain and spinal cord) of all pigs which developed spongiform encephalopathy. Infectivity was also found in the stomach, jejunum, distal ileum and pancreas but not in other tissues assayed (spleen, thymus, mesenteric lymph node, liver and kidney) of the terminally affected pigs.

However CNS tissues from terminally affected pigs failed to transmit TSE in a proportion of the challenged mice (about 10%), indicating either a relatively low titre of infectivity or, more

probably, a low sensitivity of the detection bioassay used, which raises questions about negative results obtained from some peripheral tissue bioassays.

In a second experiment, which was part of an EU project (FAIR CT97-3306), six LargeWhite x Landrace pigs aged nine weeks were inoculated intracerebrally (into the cerebral cortex) with a dose of 100 mg of BSE material each (infectious titer in RIII mice unknown). The inoculum consisted of a pool of BSE material (TSE/08/59) originating from the brainstem of 49 BSE affected cattle, supplied by the Veterinary Laboratory Agency (New Haw, Addlestone, Surrey, U.K.), homogenised in PBS at 1:10. One animal died early after inoculation from intercurrent disease, four pig were sacrificed at 400 dpi (57.1 weeks *post infection*) and one at 725 dpi (103.5 weeks *post infection*). Neither clinical nor pathological evidence of spongiform encephalopathy was found in any of the inoculated pigs. No PrP^{sc} was detected in the brains of the inoculated pigs either by immunohistochemistry or by Western blot. Moreover no infectivity was found in the brains of these pigs by intracranial inoculation into transgenic mice expressing porcine-PrP (PoPrP-Tg001 mice) (Castilla *et al.*, 2004). However, the results obtained in this study must be interpreted with caution as the observation time was too short to exclude later manifestations of the infection and the infectivity of the inoculum was probably lower than in the first study.

2.3 Experimental TSE infections in transgenic mice

Transgenic mice over-expressing (about 4-fold the level of expression in pig brain) porcine PrP (PoPrP-Tg001) were generated and characterised at CISA-INIA (Madrid, Spain) (Castilla *et al.*, 2004). The susceptibility of these mice to different prions (including BSE), after oral and intracerebral challenge, has been assessed or is under assessment

2.3.1 Oral challenge of Porcine-PrP transgenic mice with the agent of cattle BSE.

PoPrP-Tg mice were inoculated by intragastric route four times at intervals of one week with 0.1 ml of 50% brain homogenates from cattle with confirmed natural BSE. No evidence of infection was observed after 600 dpi as assessed by histopathology, Western blot and immunohistochemistry (Juan Maria Torres, personal communication). Infectivity in the brain of these mice is being assessed by subpassage in the same Po-PrPTg mice. No infectivity has been detected up to date (>600 dpi). Infectivity in other organs (e.g. spleen) was not tested (Juan Maria Torres, personal communication). The infectivity of the BSE inocula used in these experiments was shown to be efficiently transmitted (100% of attack rate and about 300 dpi of survival time) in transgenic mice expressing a level of bovine PrP (BoPrP-Tg110 mice) similar to that in the porcized transgenic mice (Castilla *et al.*, 2003).

2.3.2 Intracerebral challenge of Porcine-PrP transgenic mice with the agent of cattle BSE

Susceptibility of PoPrP-Tg mice to cattle BSE was characterized using three different cattle BSE isolates by intracranial inoculation (20 µl of 10 % brain homogenates from cattle with confirmed natural BSE) (Castilla *et al.*, 2004 and Juan Maria Torres, personal communication). The amount of infectivity in the BSE inocula used in these experiments was shown to be sufficiently high to produce a 100% attack rate and about 300 dpi of survival time in transgenic mice expressing a level of bovine PrP (BoPrP-Tg110 mice) similar to that in the porcized transgenic mice.

Pathological changes (vacuolar changes) and/or PrP^{Sc} accumulation (WB and IHC) were observed in a low proportion of individuals (<20%) after long survival times (>600 dpi).

Additional second-passage transmission experiments were conducted using brain homogenates from BSE-inoculated PoPrP-Tg mice showing either detectable PrP^{Sc} or not at the end of their lifespan. In both cases, a full transmission to PoPrP-Tg mice was observed after a substantially reduced survival time (<200 dpi). PrP^{Sc} was detectable in 100% of the inoculated mice. This proved the existence of a subclinical infection in the PrP^{Sc} negative mice after primary challenge with BSE. Moreover, this observation suggests that once adapted in Porcine BSE could be highly virulent for that species. However to date no results are available concerning:

- Oral transmissibility of the BSE adapted in PoPrP-Tg;
- Behaviour in PoPrP-Tg mice model of BSE adapted in pig

2.3.3 Intracerebral challenge of Porcine-PrP transgenic mice with the agent of sheep adapted BSE

PoPrP-Tg mice were i.c. challenged with BSE passaged in ARQ sheep. An attack rate of 100% with a survival time of 458±11 dpi was observed, indicating that the PoPrP-Tg mice are fully susceptible to BSE after passage in sheep. Secondary sub-passage in these mice showed a considerable reduction in survival time (162 ± 4 dpi) which is maintained in subsequent sub-passages. These results could suggest an increased virulence of BSE in the PoPrP-Tg mouse model after passage in sheep. An increased virulence of sheep BSE was previously reported in BoPrP-Tg mouse model (Espinosa *et al.*, 2007) suggesting that this phenomenon could occur also in the passage of BSE through other species.

2.3.4 Intracerebral challenge of Porcine-PrP transgenic mice with other TSE agents

Transmission experiments of a panel of different sheep scrapie isolates with different strain properties (SC-UCD-99, SC-LANGLADE, SC-N662-97, SC-VRQ, SC83-ARR, SC48-19K, SC13-20K, SC152-Nor98-like) were performed.

Nor98 like (Atypical scrapie) isolate was the only one able to infect PoPrP-Tg mice after i.c. inoculation (25% of attack rate with a survival time ranging from 200 to 600 dpi). Secondary passage shows 100% attack rate and 162 days survival time (Juan Maria Torres, personal communication). The other scrapie isolates included in the panel were not able to induce pathological changes in PoPrP-Tg mice after i.c. inoculation neither in primary passage nor in subsequent passages (Herva *et al.*, 2007).

2.3.5 The BSE transmission barrier to pigs

Studies on transgenic animals have clearly shown that the transmission barrier in prion diseases is to a large extent determined by the primary sequence of the PrP protein, while the exact requirements in the PrP sequence for the transmission barrier are not always fully understood and may vary from one interspecies transmission to another and from one prion strain to another one (Scott *et al.*, 2005; Nonno *et al.*, 2006; Espinosa *et al.*, 2007). Experimental data support the existence of a BSE transmission barrier to pigs. However this transmission barrier can not be quantified in the current stage of knowledge.

Data are lacking to assess the level of the transmission barrier in case BSE would derive from another species than cattle or in the context of other TSEs. However, results from experimental challenge of porcine PrP transgenic mice with ovine BSE indicate that this species barrier may be lower than the species barrier for cattle BSE.

2.3.5.1 The transmission barrier to humans for the agent of pig adapted BSE

Infectivity of prions generated after BSE transmission in PoPrP-Tg mice is being assessed in humanized transgenic mice overexpressing human PrP (M129M or V129V) recently developed in CISA-INIA. Infectivity in HuPrP-Tg mice is assessed by i.c. inoculation of 20 µl of 10 % brain homogenates from BSE adapted to PoPrP-Tg mice and the results compared to those of the original cattle BSE inoculum under the same conditions. Results showed a similar transmissibility in HuPrP-Tg mice (60-70% of attack rate with survivals times ranging from 400 to 600 dpi) for cattle BSE and BSE passaged in PoPrP-Tg mice (Juan Maria Torres, personal communication). A similar experiment is ongoing with pig adapted BSE. These results, could suggest that BSE maintains its infectivity for humans after passage in pigs. However, this model does not directly allow the quantification of the pig to human species barrier.

2.4 Natural BSE in Pigs

There are no reports available in the scientific literature describing a naturally occurring TSE in pigs. However, the significance of this observation has to be discussed with regards to the exposure of the animals to TSE agents in general and BSE in particular, and to our capacity to identify TSEs in pigs.

2.4.1 Historical exposure of pigs to TSE agents

In the UK, during the period before the BSE epidemic, the MBM used was produced from offal arising from pig, ruminant and to a lesser extent, poultry. Generally the inclusion rates of MBM in commercial feeds were usually greater than in ruminant rations. The maximum inclusion rates in feed for breeding pigs in the 1980s in the UK were 7% for MBM and MM with an average inclusion rate of 5% MBM in pig feeds (Matthews and Cooke, 2003). Pigs continued to be exposed in the UK after the introduction of the feed ban for ruminants in July 1988 (Anon., 1988) until September 1990 when legislation banned the use of specified bovine offals (SBO) including brain and spinal cord in all animal feed (Anon., 1990).

As a result of these feeding practices, pig products would have been recycled to pigs (Matthews and Cooke, 2003). With the ban on SBO in animal feed, pig material contributed in greater proportion to MBM.

2.4.2 Likelihood of identifying BSE clinically infected pigs under natural conditions

In the case of BSE, or another prion disease of pigs, there are factors that argue both for and against detection under commercial farming conditions.

A description of the clinical signs of BSE in pigs, based on an experimental infection, was published in 1990 (Dawson *et al.*, 1990).

However, the sporadic occurrence of some neurological signs, particularly ataxia and paresis, in a single animal within a herd is unlikely to arouse a BSE suspect and may result in the disposal of the animal without veterinary consultation.

One important factor that may militate against the identification of BSE or other TSEs in pigs is the relatively short lifespan of pigs in standard commercial enterprises, particularly if such diseases occurred at low incidence. Experimental data from an oral transmission study of BSE in pigs (Wells *et al.*, 2003) indicates the existence of cattle-pig species barrier, such that, in common with most interspecies transmissions of TSEs, first pass transmission under natural conditions, would result in a long incubation period.

Under those circumstances, the structure of the pig population is not favourable for the clinical identification of pig BSE.

The UK marketing system of commercially reared pigs has a pyramidal shape with the generation of elite genotypes in Nucleus Herds, the production of replacement stock in multiplier herds which are used in commercial herds to produce slaughter pigs (Source of information: Meat and Livestock Commission, 2007). The average age at slaughter of clean pigs (finished for meat) in the UK is 180 days. The pig slaughter figures for the period 1988 – 2006 indicate that 960,000 pigs survived to four years or more, 151,000 survived to five years or more and only 6,000 survived to six years or more (Table 1).

Table 1. **Number of slaughtered pigs, in thousands of heads, in UK by year and estimated age** (source of information Meat and Livestock Commission, 2007)

Year	Clean (180 days)	Total Sows/boars	Estimated n° of sows by age at slaughter		
			>= 4 years	>= 5 years	>= 6 years
1988	15423.4	383.8	51.0	9.6	0.4
1989	14212.7	331.2	44.1	8.3	0.3
1990	13878.6	324.6	43.2	8.1	0.3
1991	14091.0	365.8	48.7	9.1	0.4
1992	13956.9	369.7	49.2	9.2	0.4
1993	14254.7	355.5	47.3	8.9	0.4
1994	14680.8	388.6	51.7	9.7	0.4
1995	14021.3	354.7	47.2	8.9	0.4
1996	13897.1	323.8	43.1	8.1	0.3
1997	15132.9	362.7	48.2	9.1	0.4
1998	15678.1	411.1	54.7	10.3	0.4
1999	14349.7	378.7	50.4	9.5	0.4
2000	12370.3	321.5	42.8	8.0	0.3
2001	10446.3	179.6	23.9	4.5	0.2
2002	10260.4	314.3	41.8	7.9	0.3
2003	9133.2	240.8	32.0	6.0	0.2
2004	8972.5	235.2	31.3	5.9	0.2
2005	8970.6	202.4	26.9	5.1	0.2
2006	8900.2	196.4	26.1	4.9	0.2
Total	242630.7	6040.6	803.4	151.0	6.0

These figures indicate that only a relatively small number of pigs in the UK reach an age at which one would expect clinical signs to develop, if the incubation period in pigs is longer than for cattle.

In the UK pigs were exposed to the BSE agent and, due to the inclusion in pig rations of MBM of porcine origin, had primary infection of pigs from cattle with BSE occurred, there would have been the potential for recycling a porcine-adapted BSE agent.

Given the limitations of the surveillance and the length of the incubation time in relation to the normal commercial lifespan of the animals, up to date it cannot be excluded that cases could have occurred at a low level.

2.4.3 Possibility of spontaneously occurring TSE in pigs

The possibility for a spontaneously occurring TSE has been usually assumed mainly in humans as sporadic Creutzfeldt–Jakob disease (sCJD). The mechanism by which PrP^{Sc} molecules and infectious prions originate in spontaneous TSE like sCJD is not known. PrP^{Sc} molecules invariably accumulate in the brains of patients with sCJD, and the disease is transmissible. Several hypotheses have been proposed to explain the occurrence of sCJD, including: stochastic formation of PrP^{Sc} molecules, somatic mutation of PrP sequence in individual brain cells, and age-dependent decline in PrP^{Sc} clearance mechanisms.

Spontaneous TSEs in animals has not been confirmed in nature. However, such theoretical possibility can not be excluded.

Recent results show that interactions between PrP^C molecules and endogenous polyanions led to the spontaneous formation of infectious prions in unseeded Protein Misfolding Cyclic Amplification (PMCA) experiments (Atarashi *et al.*, 2007; Deleault *et al.*, 2007). Moreover, experiments show the spontaneous generation of PrP^{Sc} and infectious prions in unseeded PMCA experiments using brain homogenate from healthy bank voles (Castilla *et al.*, 2007).

2.4.4 TSE Surveillance in pigs

Under Regulation (EC) No 999/2001 (EC, 2001) Member States may, on a voluntary basis, carry out monitoring for TSE in animal species other than bovine, ovine and caprine animals.

Systematic TSE surveillance in pigs has never been carried out.

In 1997 an incident from 1979 was reported in the USA in which slaughtered pigs seemed to show neurological signs and microscopic evidence of encephalopathy (Hansen and Halloran, 1997). In one pig out of 60 examined, neuronal vacuolation and gliosis were found. The affected pig was 6 months old. Subsequent re-examination of the material showed that the lesions were not pathognomonic of those seen in TSEs (G. A. H. Wells, personal communication).

Localised vacuolar changes, particularly vacuolation of neuronal perikarya in certain brain nuclei, is occasionally observed as an incidental finding in many species, including pigs and appears to be unrelated to TSE/prion disease, or other known systemic disorders.

Small scale testing surveys in pigs, utilising PrP^{Sc} rapid testing, have been undertaken in some countries:

- In Ireland a survey for TSEs in pigs fed meat and bone meal failed to identify any evidence of TSEs in a sample of 1107 adult pigs which were approximately three-and-a-half years old (Jahns *et al.*, 2006);
- In Switzerland a pilot study for TSE surveillance investigated 372 slaughtered and 63 fallen stock adult pigs by histopathology, IHC and BioRad ELISA. Some animals showed mild histopathological changes, but none was positive on either IHC or BioRad ELISA (Kofler *et al.*, 2006).

However, due to the low sample number and the unknown age of the tested pigs, these data are inadequate to reliably exclude the presence of natural TSE infections in pigs.

2.4.5 Risk of residual infectivity after exposure to BSE contaminated feed

In a previous SSC opinion on the potential requirement for designation of specified risk materials (SRM) in pigs. (SSC, 2003) the following reasoning was developed:

A separate, but real, concern in countries where feed controls are restricted to feed manufactured for consumption by ruminants, is that pigs could, prior to slaughter have been fed BSE contaminated feed and that without the pig becoming infected, the infectivity in the intestinal lumen could be transferred to feed via rendering (Matthews and Cooke, 2003). As a result, these animals could perpetuate cycles of transmission through feed and thereby undermine the effectiveness of feed bans. For example, where ruminant protein may still be fed to pigs and poultry, their offal may still represent a risk of recycling infectivity to ruminants if intestinal contents are still present at the time of rendering. In other words, if ruminant protein may be fed to pigs and porcine MBM may still be fed to ruminants, the intestine of the pig at slaughter, and consequently the porcine MBM, may contain ruminant protein. Clearly, under current regulations, this eventuality is prevented within member states of the EU.

The same reasoning was followed in the EFSA opinion on “The assessment of the health risks of feeding of ruminants with fishmeal in relation to the risk of TSE” (EFSA, 2007), concluding that “*If there is any risk of TSE in fishmeal, this could arise from the mammalian feed being fed to this fish or through fishmeal contaminated by MBM*” and “*there is a potential hazard of residual TSE infectivity in fishmeal produced from fish recently fed with TSE contaminated feed.*”

Consequently there is a hazard of residual BSE infectivity in pigs and pig PAPs produced from pigs recently fed with BSE contaminated feed. The feed ban implemented in 2001 made it highly unlikely that pigs were since this date exposed to significant amounts of BSE infectivity through feed.

2.5 TSE Infection in pigs: conclusion on the current scientific information

- Data available on pigs in relation to TSEs remains sparse, which limits the possibilities to realize TSE risk assessment in this species.
- There is clear experimental evidence that pigs are susceptible to BSE when parenterally exposed. However, within the limits of the only pertinent available studies (size of animal groups and investigation methods), no transmission (clinical disease or PrP^{sc} accumulation) was observed after BSE oral challenge in this species.

- According to experimental work carried out in PoPrP-Tg mice, modelling the different transmission barriers, BSE agent after adaptation in porcine seems to acquire a high virulence for this transgenic species and to conserve its capacities to cross species barrier including human transgenic mice.
- It is possible that there is a hazard of residual BSE infectivity in pigs and pig PAPs produced from pigs recently fed with BSE contaminated feed. However, with the feed ban in place since 2001, it is highly unlikely that pigs are presently being fed with considerable amounts of BSE infectivity.
- No evidence exists of naturally occurring TSEs, including BSE, in pigs. However, given the limitations of the surveillance and the length of the incubation time in relation to the normal commercial lifespan of the animals, up to date it cannot be excluded that cases could have occurred at a low level.

2.6 *BSE infection in birds*

Because the low homology (30%) existing between the mammalian PrP gene, that has been shown to play a key role into TSE susceptibility, and the PrP gene in birds, the probabilities for a mammalian prion to propagate in birds are considered to be very low.

Experiments aiming at establishing potential susceptibility of birds to BSE are limited. The most relevant published experiment included experimental challenge in chicken with a BSE cattle inoculum prepared from the brainstem of terminally affected cattle, but harbouring an unknown infectious titer (Matthews and Cooke, 2003):

- 12 chickens aged one day were i.c. challenged with 50µl of a 10% saline suspension of pooled brain stem. A further 1ml was inoculated i/p when the chickens were 2 weeks old (about 10^4 i.c./i.p. mouse LD₅₀).
- 11 chickens were orally challenged with a total of 5g of a pool of brain tissue from two cattle with confirmed BSE on three occasions when the birds were 4, 5, and 6 weeks of age. The material was deposited in the distal oesophagus/crop.

No evidence of spongiform encephalopathy was found at the conclusion of the study. Sub-passages were performed in conventional mice models using central nervous tissue of inoculated chickens, with no evidence of transmission.

However, it should be noted that:

- a. a validated TSE diagnosis tool (method to detect abnormal avian prion protein accumulation) is lacking;
- b. potential species barriers between chicken and mice could impair infectivity detection through bioassay.

In conclusion, up to date there is no evidence that poultry are susceptible to BSE.

The possibility that poultry act, after oral challenge under field conditions, as healthy silent carriers in the spread of the BSE agent is still hypothetical and no results of experiments conducted as yet are available to support this hypothesis.

However, available experimental data rely on a very limited number of animals.

The only hazard that can be identified is residual BSE infectivity in poultry and poultry PAPs produced from poultry recently fed with BSE contaminated feed.

2.7 Current discriminatory tests for products containing animal proteins in feed

Pure animal proteins are rarely used as such in feedingstuffs. It is thus easier to speak about products containing animal proteins. It is also in this spirit that restrictions are considered in Art. 1, point 7.) of Reg. (CE) 1923/2006 (EC, 2006) amending Reg. 999/2001. Furthermore, this is in line with the fact that with current technology a tolerance level would have to be defined as a maximum mass fraction of prohibited products containing animal proteins, rather than in quantities of animal proteins themselves.

In this section the products containing animal proteins encompass all animal products with a sufficient amount of proteins (e.g. pure animal fats are thus excluded) without considering however for prohibition or for the definition of a tolerance level those products that are explicitly authorized in annex IV of regulation 999/2001 (e.g. milk, eggs or egg products,...).

The target animal products also include processed materials that pose a specific challenge for some of the currently applied methods such as immunoassays and PCR.

With respect to the terms of reference of the mandate, two points have to be highlighted concerning the state of the art of the detection methods:

- 1) Is species-specific detection possible in order to avoid intra-species recycling?
- 2) Is it possible with present-day available methods to determine with sufficient confidence if a set tolerance level has been exceeded or not?

Different techniques are currently applied for the detection of products containing animal proteins in feed and the following methods are most often used:

- (i) Classical Microscopy;
- (ii) Microscopy coupled to Near Infrared, which is defined as Near Infrared Microspectrometry (NIRM);
- (iii) Immunoassay;
- (iv) Polymerase Chain Reaction (PCR).

None of the previous methods measure animal proteins "as such", but they detect specific "targets" indicating their presence.

In fact, classical microscopy and NIRM focus mainly on bones, whereas immunoassays and PCR measure specific proteins and DNA targets, respectively. This aspect has major consequences for the performance profile of the methods, which will be presented and compared in this section, while a summarized overview is given in Table 2.

Classical microscopy is the official method (EC, 2003) by which the correct implementation of the European regulation concerning the feed ban is checked. The controls, performed by the National EU MS Official Laboratories, are audited by the Food and Veterinary Office of the European Commission. The results of the control programmes in the EU Member States are summarized in Appendix B.

Table 2: Overview of the characteristics of the various analytical methods, main features of Classical Microscopy, Near Infrared Microspectrometry (NIRM), Immunoassay methods and Polymerase Chain Reaction (PCR) in the detection of products containing animal proteins in compound feed adapted from Baeten *et al.*, 2005b.

	Classical Microscopy	NIRM	Immunoassay	PCR
Analytical features				
Samples/day	10–15	3–5	100–200	5–10
Analytical time/sample	45–60 min	2 hours	30 min	2 days
Sampling	5–10 g	0.2–10 g	10 g	0.1– 40 g
Limit Of Detection (LOD)	≤ 0.1%	≤ 0.1%	~ 0.5%	~ 0.1%
Transferability ¹	high	high	high	medium
Matrix dependent	no	no	yes	yes
Animal tissue dependent	yes	?	yes	no
Species or species-group discrimination	limited	?	yes	yes
Interfering ingredients or processes				
Other authorized products (milk, blood, eggs,...)	no	no	yes	yes
Fat	no	no	yes	yes
Heat -treated material	no	no	no <141°C	no <141°C
Particle size	no	partly	no	no
Miscellaneous				
Validated method	yes	yes	yes ²	yes ²
Existing facilities	yes	no	yes	yes
Quantitative method	yes	yes	no	no

¹ Transferability is the successful application of the method by other laboratories that were not involved in the development of the protocol

² At least for some targets

2.7.1 Species-specific detection

2.7.1.1 *Classical microscopy and Near Infrared Microspectrometry (NIRM)*

Currently the official method for detection of products containing animal proteins, including MBM, in feed is classical microscopy as specified in Commission Directive 2003/126/EC (EC, 2003). The method as such is unable to identify the species of origin of a product containing animal proteins. Analysis by classical microscopy of products containing animal proteins available on the European market can only make the difference between fishmeal and MBM of animals of terrestrial origin (Raamsdonk *et al.*, 2004). However, it should be stressed that research is ongoing to try to differentiate between avian and mammalian material. Based on some bone fragment characteristics especially linked to the lacunae inside

the bone fragments, some descriptors that generally differentiate a bone of avian origin from one of mammalian origin have been found (Pinotti *et al.*, 2004). Nevertheless, this seems only a very general trend and an avian bone might sometimes have the appearance of one of mammalian origin depending from which bone it comes and its growth characteristics (Mondini *et al.*, 1999). Another potential criterion of distinction within groups of species of terrestrial animals might be linked to the density of striae in muscle fibres (Raamsdonk *et al.*, 2005). NIRM methods are also unable, at present, to distinguish more than classical microscopy between groups of animals even if there seem to be some potentialities with spectral analysis to identify up to species level or maybe groups of species (De la Haba *et al.*, 2007; Fumière *et al.*, 2007).

2.7.1.2 Immunoassay

Immunoassays represent one of the techniques that are able to identify, up to species level, the origin of a product containing animal proteins.

As ruminants and cattle, in particular, were prominent in TSE issues, most tests were focussed on these species or group of species and among them some methods appear to be appropriate for detection of 0.1 % of MBM in feed as shown by an independent inter-laboratory study (Boix *et al.*, 2004; Prado *et al.*, 2006). These results demonstrate the significant improvement of the sensitivity of immunoassays compared to formerly used tests, which is mainly due to the fact that the more sensitive tests utilise heat stable proteins as antigen. Another laboratory (CCL, Veghel, The Netherlands) also tested various immunological techniques (S. van den Hoven and R. Margry, personal communication) obtaining similar results, thus supporting the results from the above mentioned inter-laboratory study.

The sensitivity of the species-specific detection of animal proteins in compound feedingstuffs⁹ is significantly better compared to the detection of the same amount of animal proteins in a mixture of high amounts of animal proteins of other species.

Some authorised products such as dicalcium phosphate might also contain traces of the target proteins, which could lead to a positive response of the immunoassay. Such a response would therefore erroneously indicate the presence of banned products containing animal proteins. Another limitation is related to the fact that the selected proteins can be highly dependent on the tissue composition of the product containing animal proteins. For instance, the target troponin I can only be found in striated muscle.

2.7.1.3 PCR

In principle, PCR is capable of differentiating a product containing ruminant DNA from one containing non-ruminant DNA or, more generally, even to identify its species composition, thus fulfilling the criteria for a species-specific detection. Some important aspects need to be taken into account when developing PCR methods for this specific purpose, especially due to the different heat treatment that products containing animal proteins have undergone. In fact, an inter-laboratory study carried out in 2003 (Gizzi *et al.*, 2004) showed that almost all

⁹ Compound feedingstuffs: organic or inorganic substances in mixtures, whether or not containing additives, for oral animal feeding in the form of complete feedingstuffs or complementary feedingstuffs (Council Directive 79/373/EEC)

cattle/ruminant specific PCR methods failed to detect in animal feed a level of 0.1 % MBM treated according to EU legislation. Since that time various PCR methods (e.g. Fumière *et al.*, 2006) have been developed showing a sensitivity at the level of 0.1%, as demonstrated in a recent inter-laboratory study (Prado *et al.*, 2006). In particular these methods address the specific challenge of the effect of the rendering on the length of the target DNA. In this study three laboratories applied successfully their own PCR detection method for ruminant or bovine targets on the same test samples. The level of heat treated products containing animal proteins in those samples was 0.1 %.

In addition, other animal species were also considered. For instance the pig target developed at CRA-W has given successful results in several proficiency tests (Fumière *et al.*, 2006) in which feed with PAPs were analysed. Other institutions (e.g. TNO, Zeist, The Netherlands; VLA, Luddington, UK) developed valuable targets for pig, chickens and other species. Most of these techniques have been exclusively single-laboratory validated for species identification. Further testing – preferably by another laboratory – is strongly recommended to confirm the suitability of these methods for the intended purpose.

A major limitation of PCR is that it is unable to differentiate DNA of unauthorized products from that of authorized ones (e.g. egg products or animal fat). This means that when analysing compound feed samples, a positive PCR signal could be erroneously interpreted as proof for the presence of banned products containing animal proteins. This problem is of minor importance when analysing product containing animal proteins as a raw material originating from a single species (or group of species like poultry) or pure feed ingredients because there is no reason to find, for instance, egg products in porcine MBM. A future perspective for that kind of problem of the species identification are the coming results from current research projects like the FP6 SAFEED-PAP project (<http://safeedpap.feedsafety.org/>).

2.7.2 Tolerance level

When considering the setting of a tolerance level, the performance of each methods for a quantitative estimate of products containing animal proteins in feed need to be elaborated. As already pointed out, the unknown processing conditions of a product containing animal proteins present at traces level in feed samples have a strong impact on the response of immunoassay and PCR methods. In consequence, these methods can not be utilised for a quantification of the ratio of products containing animal proteins in this matrix. In contrast, methods based on microscopy have, in principle, the potential for a quantitative estimation and are therefore exclusively considered in this chapter.

This also means that the unit of expression of the tolerance level is not an amount of protein per weight of feed but a maximum mass fraction of prohibited products containing animal proteins (including those that might result from contamination, e.g. presence of traces of rodent material).

With the existing total feed ban, the detection of infringements is only possible for products containing animal proteins at levels that are at or above the Limit Of Detection (LOD) of the microscopic method.

From a detection viewpoint a tolerance level should be defined in such a way that technically it is feasible to determine, with some confidence, if this level is exceeded or not. Practically this means that a quantitative detection is required and therefore the level should at least be set above the Limit of Quantification (LOQ) of the methods for instance by a factor of 2 to

stay within an acceptable range for the uncertainty of the measurement. In that perspective, it might be of interest to list some accepted definitions of LOD and LOQ.

- **Definitions of the “Limit of Detection” (LOD)**

“The amount of an analyte corresponding to the lowest measurement signal which with a defined confidence may be interpreted as indicating that the analyte is present in the test sample, but without allowing quantitation.” (Codex Alimentarius, 2007)

- **Definitions of the “Limit of Quantification” (LOQ)**

“The limit of quantification (LOQ) (also called limit of determination) of an analytical procedure is the lowest amount of analyte in a laboratory sample which can be quantitatively determined with a defined confidence.” (Codex Alimentarius, 2007)

If a tolerance level would be defined for presence of products containing animal proteins in general, whatever their species origin, then classical microscopy seems for the moment the most appropriate technique to determine if this level is exceeded or not.

2.7.2.1 Classical microscopy and Near Infrared Microspectrometry (NIRM)

The official microscopic method, Commission Directive 2003/126/EC (EC, 2003), foresees the option of estimating the amount of animal constituents in feed. According to the above mentioned directive "very small amounts of constituents of animal origin (< 0,1 %) in feedingstuffs can be detected". Comparing this statement with the above mentioned definition of LOD and LOQ indicates that the value of 0.1 % defines the limit of detection, whereas a LOQ is not given. The Commission Directive clarifies that the actual sensitivity depends on the nature of the constituents of animal origin. This aspect reflects a major restriction when applying microscopic method, because the probability to detect traces of products containing animal proteins depends largely on its bone content. In fact, if the bone fraction of the specific product containing animal proteins present in the feed is low, the probability to detect these traces is lowered, thus the method becomes less sensitive. If moreover there are high amounts of fishmeal present, this may have a masking effect (Gizzi *et al.*, 2004) leading to a LOD that can exceed 0.1% in some cases. On the other hand, the specific rendering conditions underwent by a product containing animal proteins have only a minor impact on the performance profile of microscopic methods.

The IAG (“*Internationale Arbeitsgemeinschaft für Futtermitteluntersuchung*”, which is the German denomination for International Association for Feedingstuff Analysis) is already setting up quantitative tests to assess the proficiency of laboratories with, however, very variable results (<http://www.iag-micro.org/index.php>).

In a recent inter-laboratory trial organized by the CRL-AP (Veys and Baeten, 2007) laboratories had to apply classical microscopy to quantify fishmeal in feed. The results of the study indicate that, at present, the method is not yet fit for this specific purpose. However, the results also show that possibilities are realistic to improve it and to reach an unbiased figure of the content of fishmeal in which confidence can be put, taking into account an acceptable variability. Nevertheless, the precise performance of this updated and harmonised procedure should be assessed to be able to make a clear statement about its actual possibilities of quantification by classical microscopy.

Near infrared microspectrometry methods seem also very suitable for a quantitative purpose when applied on the sediment (Baeten *et al.*, 2005a) with the advantage to be more independent of the analyst. Transferability of the method has been recently confirmed by successfully applying the protocol in two other laboratories that were not involved in the development of the method (Boix *et al.* 2007). However, as the equipment required is not yet widely spread, it was impossible until now to carry out a validation study through an inter-laboratory trial.

As already mentioned the official reference method is essentially focussed on bone spicules, therefore when there are no bones or almost no bones in the products containing animal proteins, a situation that is not merely theoretical, although probably not that frequent, with products containing animal proteins that derive from the so-called “soft tissues” (consisting essentially of intestines but with apparently always a low bone content) the quantification by the official method remains a still unexplored matter. In these cases microscopy should still be able to perform detection at least on the raw fraction but a higher limit of detection is to be expected.

When fixing a threshold as tolerance level, it is necessary that the kind of consensus limit of quantification (LOQ), that would be valid for a large range of products containing animal proteins, is well below this threshold limit.

It is currently not possible to set a LOQ because of insufficient studies conducted up to now on the performance of relevant detection methods for quantification.

The possible measurement errors should consider the entire analytical process which means not only that of the analytical steps but also the sampling scheme. For time being there is however no reason to suspect that the sampling scheme proposed by directive 76/371/EEC (EEC, 1976) is not valid and therefore focus should essentially be put on the analytical aspects.

2.7.3 Limitation of setting tolerance levels in the event of poultry PAPs being allowed to be fed to pigs and vice versa

A last point to consider are the implications, in terms of detection of cross-contamination in feed, that could arise from a policy of lifting the ban on the use of poultry PAPs for pigs and *vice-versa* and simultaneously fixing a tolerance level for unauthorised products containing animal proteins in the same feed.

For instance, feed for poultry containing authorised proteins from pigs could also contain proteins from other animals (including ruminants). Enforcing tolerance levels for traces of unauthorised products containing animal proteins in this type of feed would therefore require analytical methods that allow for a species specific and quantitative determination of these unauthorised products.

As shown above, such a method does not exist yet. In fact, microscopy would deliver a quantitative estimate of products containing animal proteins in general, but without differentiating between the species origin. In contrast, PCR would allow for the detection of traces of products containing animal proteins from prohibited species, but without giving a quantitative estimate.

3 Risk Assessment

3.1 *BSE Risk deriving from the utilization of poultry PAPs in pig feed*

Even recognising that significant amounts of BSE infectivity have been fed to pigs in the UK and additionally intra-species pig to pig recycling have happened, no naturally occurring TSE, including BSE, have been detected so far in pigs. It is therefore concluded that if TSE was present in pigs it would not have been at an epidemic level. However, major limitations of available data do not allow conclusive evidence of the absence of TSE in pigs. In this context intra-species recycling in pigs continues to be a hazard.

Excluding the scenario of pig-to-pig intra-species recycling (but allowing some very limited recycling through poultry PAPs from poultry previously fed with pig PAPs, as poultry intestines are not washed during rendering), the risk of transmitting BSE to pigs utilizing poultry PAPs is negligible, as there is no evidence that poultry are susceptible to BSE infection.

The only two hazards that can be identified are:

- residual BSE infectivity in poultry and poultry PAPs produced from poultry recently fed with BSE contaminated feed
- cross-contamination of poultry PAPs with BSE contaminated material

Considering the current epidemiological situation of BSE in the EU and the current control measures in place to avoid the exposure of poultry to BSE contaminated material, these two sources of contamination could be considered to be a negligible risk of exposing pigs to BSE. Consequently in this scenario the increase in the exposure risk of BSE to humans, if any, would be negligible.

In the hypothesis of identification of TSE in birds under natural conditions this assessment should be reconsidered in particular with regard to the risk of recycling poultry TSE in poultry through the use of pigs that could have been exposed to poultry TSE contaminated material.

It is noteworthy to underline that such assessment remains valid only in the context of the continuation of the other regulatory measures laid down in Reg. EC 999/2001 and 1774/2002 (ban on intra-species recycling, SRM removal and ban on proteins derived from ruminants).

Moreover, if pigs were fed with TSE-contaminated material the content of the gut and manure may present a risk because even the treatment at “133°C/20’/3 bars” may not eliminate all TSE infectivity if the initial titre was high.

3.2 *BSE Risk deriving from the utilization of pig PAPs in poultry feed*

According to the current knowledge the risk of transmitting BSE to birds is considered negligible. Moreover, there is no evidence of active replication of the BSE agent in birds after oral exposure. However, limitations of available data do not allow us to definitely conclude that TSE is absent in birds.

Excluding the scenario of poultry-to-poultry intra-species recycling (but allowing some very limited recycling through pig PAPs from pigs previously fed with poultry PAPs), the risk of transmitting BSE to poultry utilizing pig PAPs is negligible, as long as there is no evidence of naturally occurring TSE in pigs.

The only two hazards that can be identified are:

- residual BSE infectivity in pigs and pig PAPs produced from pig recently fed with BSE contaminated feed
- cross-contamination of pig PAPs with BSE contaminated material

Considering the current epidemiological situation of BSE in the EU and the current control measures in place to avoid the exposure of pigs to BSE contaminated material, these two sources of contamination could be considered to be a negligible risk of exposing poultry to BSE. Consequently in this scenario the increase in the exposure risk of BSE to humans, if any, would be negligible.

In the hypothesis of identification of TSE in pigs under natural conditions this assessment should be reconsidered in particular with regard to the risk of recycling pig TSE in pig through the use of poultry that could have been exposed to pig TSE contaminated material.

It is noteworthy to underline that such assessment remains valid only in the context of the continuation of the other regulatory measures laid down in Reg. EC 999/2001 and 1774/2002 (ban on intra-species recycling, SRM removal and ban on proteins derived from ruminants).

Moreover, if poultry were fed with TSE-contaminated material the content of the gut and manure may present a risk because even the treatment at “133°C/20’/3 bars” may not eliminate all TSE infectivity if the initial titre was high.

3.3 Risk of transmitting BSE through small quantities of products containing animal proteins in feed

3.3.1 Risk of transmitting BSE through small quantities of products containing animal proteins in feed to ruminants

Regarding the inclusion of animal proteins in ruminant feed a quantitative risk assessment has been described in the EFSA opinion “Quantitative risk assessment of the animal BSE risk posed by meat and bone meal with respect to the residual BSE risk ” (EFSA, 2005).

One example, representative for the present situation in EU, was that, under the scenario of feeding cattle 2-3 kg per day of compound feed containing 0.1% MBM with a 40% bovine origin from a SSC GBR III country¹⁰ with reliable surveillance and all SRM removed, the average exposure is $1,2 \times 10^{-7}$ Co ID₅₀ (Cattle oral Infectious Dose) per year and that the 97.5 percentile is 7.8×10^{-7} . This means a 40 to 1 against chance that the exposure would exceed 7.8×10^{-7} . At this level it is very unlikely that an animal would become infected. Assuming a linear-dose response curve at very low dose this would be equivalent to a few animals per year in the EU cattle population: 1 infected animal per 10,000,000 cattle.

The worst case example concerns cattle, in an intensive system, obtaining about 8 kg of compound feed containing 0.1% MBM with a 40% bovine origin from a SSC GBRIV country¹¹ with unreliable surveillance and where no SRM was removed prior to rendering. In

¹⁰ A country in which the presence of BSE infected cattle is likely or, if already cases were discovered, the number of BSE cases identified during the last 12 months is below 100 cases per million adult cattle.

¹¹ A country in which more than 100 BSE cases per million adult cattle were discovered in the last 12 months.

that case, cattle could be exposed to a median of 5×10^{-5} CoID₅₀. These values are consistent with the extent of the BSE epidemic in the UK.

The previous two examples demonstrate that the same level of contamination could lead to different BSE risks, according to the scenario in which this contamination is considered. This is due to the interaction between the contamination and the stability of the challenged system. The stability of the system can be defined as ability of a system to avoid the propagation and amplification of the BSE agent in a country in a given time period. This mechanism is described in the EFSA Opinion on the revision of the Geographical BSE risk assessment methodology (EFSA, 2007).

Nonetheless, even in the first example, transmission of BSE, although at a very low level, cannot be excluded. Therefore the transmission of BSE through small quantities of products containing animal proteins, even under the level of 0.1%, in feed to ruminants cannot be excluded. Considering the current EU context the few infected animals that could arise from this contamination would not be able to sustain the BSE epidemic but would potentially increase the human exposure risk to BSE.

3.3.2 Risk of transmitting BSE through small quantities of products containing animal proteins in feed to non-ruminants

As in practice, the current risk assessment concerns only relatively small amounts of products containing animal proteins in feed for non ruminant farmed animals, which have not been shown to be significantly susceptible to BSE by oral route, the risk of transmitting BSE to these animals is considered to be lower than in ruminants as long as intra-species recycling is avoided. Consequently in this scenario the increase in the exposure risk of BSE to humans, if any, would be negligible.

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APPENDIX B

Results of the control programmes on the feed ban in the EU Member States

Percentage of infringements on the feed ban in the Member States (source European Commission)

Year	Ruminant feed		Non-ruminant feed		Feed materials*	
	N° Samples	% infr.	N° Samples	% infr.	N° Samples	% infr.
2001	24102	2.7 %	14751	2.7 %	2315	1.5 %
2002	26106	0.12 %	17053	0.56 %	7910	0.35 %
2003	19112	0.21 %	15410	0.47 %	10049	0.17 %
2004	24793	0.36 %	20474	1.41 %	16300	0.58 %
2005	13008	0.17 %	9393	0.80 %	5909	0.85 %

* Feed materials: “various products of vegetable or animal origin, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing additives, which are intended for use in oral animal feeding either directly as such, or after processing, in the preparation of compound feedingstuffs or as carriers of premixtures”. Article 2, Directive 96/25/EC (EU, 1996).

Explanatory note to the results

- A significant number of the infringements were duplicates (from additional samples collected after 1 positive analysis) or rather infringements on labelling (fishmeal detected in non-ruminant feed which is not prohibited but was not marked on the label). The real percentage of infringements may therefore be lower. Member States were also requested to target the controls which may have increased the percentage of infringements.
- Since the start of the extended feed ban (1 January 2001) in the European Union, the percentage of infringements continuously decreased. The slight increase in 2004 is mainly due to the amounts of remnants of meat-and-bone meal in production lines not systematically cleaned in the new Member States following Accession in May 2004.
- The results indicate that the number of infringements despite the very strict legal provisions at every stage (transport, storage, feed production, farms) does not become zero but remains at a very low level. This can be most likely explained by the adventitious presence of animal constituents in certain raw materials such as sugar beet pulp and fat and the presence of cadavers of rodents and birds in raw materials.

References

EU 1996. Council Directive 96/25/EC of 29 April 1996 on the circulation of feed materials, amending Directives 70/524/EEC, 74/63/EEC, 82/471/EEC and 93/74/EEC and repealing Directive 77/101/EEC. *Official Journal of the European Union* 125: 35 - 58.

GLOSSARY

Term	Definition
Bovine Spongiform Encephalopathy (BSE)	A transmissible spongiform encephalopathy (see below) of adult cattle. Contamination of MBM in feed with prions is considered to have caused the BSE epidemic that originated in the UK.
Cattle oral ID₅₀ (CoID₅₀)	The oral dose which infects 50% of cattle in an experimental test.
Compound feedingstuffs	In Council Directive 79/373/EEC of 2 April 1979 are defined as: <i>“Organic or inorganic substances in mixtures, whether or not containing additives, for oral animal feeding in the form of complete feedingstuffs or complementary feedingstuffs”</i>
Infectious Dose 50% (ID₅₀)	The dose which infects 50% of animals in an experimental test.
Lethal Dose 50% (LD₅₀)	The dose which kills 50% of animals in an experimental test.
Meat and Bone Meal (MBM)	In Commission Directive 92/87/EEC of 26 October 1992 is defined as: <i>“Product obtained by heating, drying and grinding whole or parts of warm-blooded land animals from which the fat may have been partially extracted or physically removed. The product must be substantially free of hooves, horn, bristle, hair and feathers, as well as digestive tract content. (1) Products containing more than 13 % fat in the dry matter must be named as 'rich in fat’”</i> . It is used as a protein source in animal feed.
Meat Meal (MM)	In Commission Directive 92/87/EEC of 26 October 1992 is defined as: <i>“Product obtained by heating, drying and grinding whole or parts of warm-blooded land animals from which the fat may have been partially extracted or physically removed. The product must be substantially free of hooves, horn, bristle, hair and feathers, as well as digestive tract content. (Minimum crude protein content 50 % on a dry matter basis)”</i> . It is used as a protein source in animal feed.
Prion	Neologism for “proteinaceous infectious particle”, frequently used as designation for the infectious agent of TSEs (see below). All known prions contain misfolded isomers of a normal cellular protein (PrP ^c). Aggregates of the misfolded protein of sufficient quantity and size are usually associated with TSE infectivity and neurodegenerative diseases in both animals and humans. According to the methodology used for detection of the disease associated, misfolded protein, different terms have been used for its destination (see below). Currently the preponderant hypothesis concerning prions considers that the misfolded protein is the only component of the infectious agent of TSEs. However, a part of TSE experts believe that the protein-only theory has not been proven beyond question.

Term	Definition
Processed Animal Protein (PAP)	In Commission Regulation (EC) No 829/2007 of 28 June 2007 is defined as: “ <i>animal protein derived entirely from Category 3 material, which have been treated in accordance with Chapter II of Annex VII so as to render them suitable for direct use as feed material or for any other use in feedingstuffs, including petfood, or for use in organic fertilisers or soil improvers; however, it does not include blood products, milk, milk-based products, colostrum, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, tricalcium phosphate and collagen</i> ”. It comprises MBM and MM (see above). It is used as a protein source in animal feed.
PrP^d	Disease associated, abnormally folded prion protein. Sometimes this acronym is used when methods for detection of disease-associated PrP are employed that are not based on proteinase resistance nor infectivity assays, such as immunohistochemistry.
PrP^{res}	Abnormally folded prion protein that is highly resistant to proteinase K digestion and is strongly associated with prion disease. This acronym is used when methods for detection of disease-associated PrP are employed that are based on proteinase resistance, such as immunoblotting or ELISA. It is sometimes used synonymously with PrP ^{sc} .
PrP^{sc}	Term originally derived from scrapie associated PrP, but also more generally used in all TSEs. Abnormally folded prion protein that has a gradient of resistance to proteinase K digestion. It is associated with infectious potential and with prion disease even in circumstances where it may be sensitive to proteinase K digestion.
PrP^{TSE}	TSE associated, abnormally folded protein. It is used synonymously with PrP ^{sc} or PrP ^d . Sometimes “TSE” is replaced by the acronym of the respective disease, e.g. PrP ^{CJD} , PrP ^{GSS} , PrP ^{BSE} , PrP ^{sc} , PrP ^{CWD} , etc
Render (rendering process)	Processing animal by-products to make MBM, MM (see above) and tallow. This is achieved by drying/cooking and separating the solid fraction (protein meals) from the melted liquid fraction (tallow and animal fat).
Scrapie	Designates a natural transmissible spongiform encephalopathy (see below) of sheep and goats. This term covers a large variety of agents (TSE strains) with different biological properties. Scrapie has been described in many parts of the world. Can be transmitted naturally or experimentally to other animals such as mice. Is the main prion source for experimental models of TSEs.

Term	Definition
Specified Risk Material (SRM)	<p>The following tissues of animals whose origin is in a Member State or third country or of one of their region with a controlled or undetermined BSE risk according to the Reg. CE 999/2001</p> <p>As regards bovine animals:</p> <ul style="list-style-type: none"> (i) the skull excluding the mandible and including the brain and eyes, and the spinal cord of animals aged over 12 months; (ii) the vertebral column excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the median sacral crest and wings of the sacrum, but including the dorsal root ganglia of animals aged over 24 months; and (iii) the tonsils, the intestines from the duodenum to the rectum and the mesentery of animals of all ages. <p>As regards ovine and caprine animals:</p> <ul style="list-style-type: none"> (i) the skull including the brain and eyes, the tonsils and the spinal cord of animals aged over 12 months or which have a permanent incisor erupted through the gum, and (ii) the spleen and ileum of animals of all ages.
Transmissible Spongiform Encephalopathy (TSE)	<p>A family of slowly progressive and ultimately fatal diseases of the central nervous system. They are characterized by transmissibility with a long incubation period, and spongiform degeneration of the central nervous system without inflammation and immunity response. Examples in humans include CJD and kuru. Among animals: scrapie and BSE. A synonym for TSE is prion disease.</p>